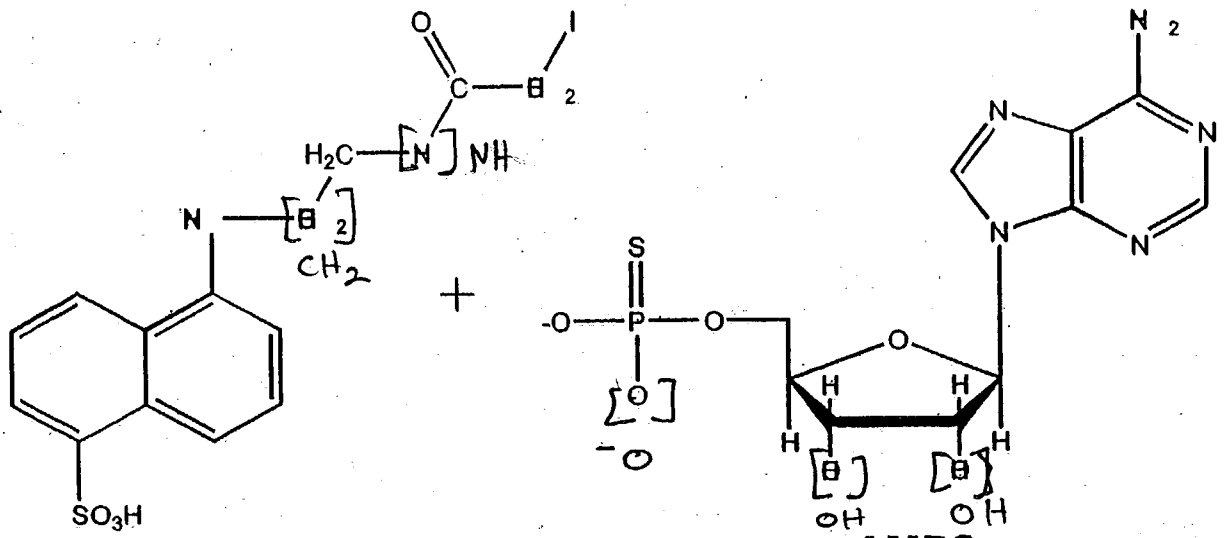
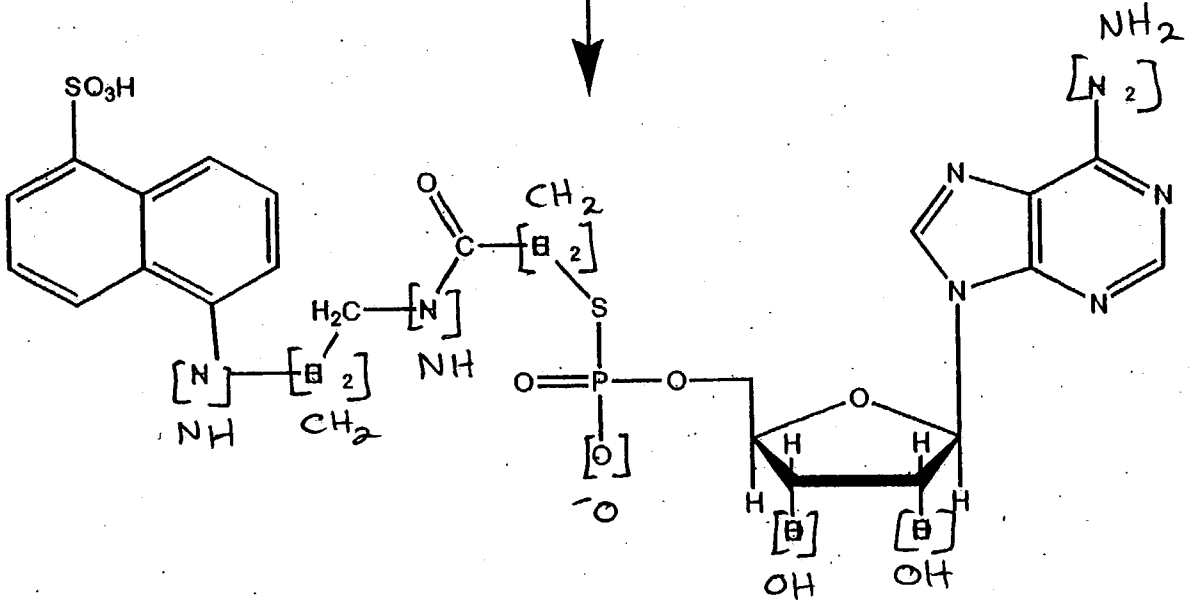


IAEDANS AMPS



5' AEDANS-S-AMP

FIGURE 3

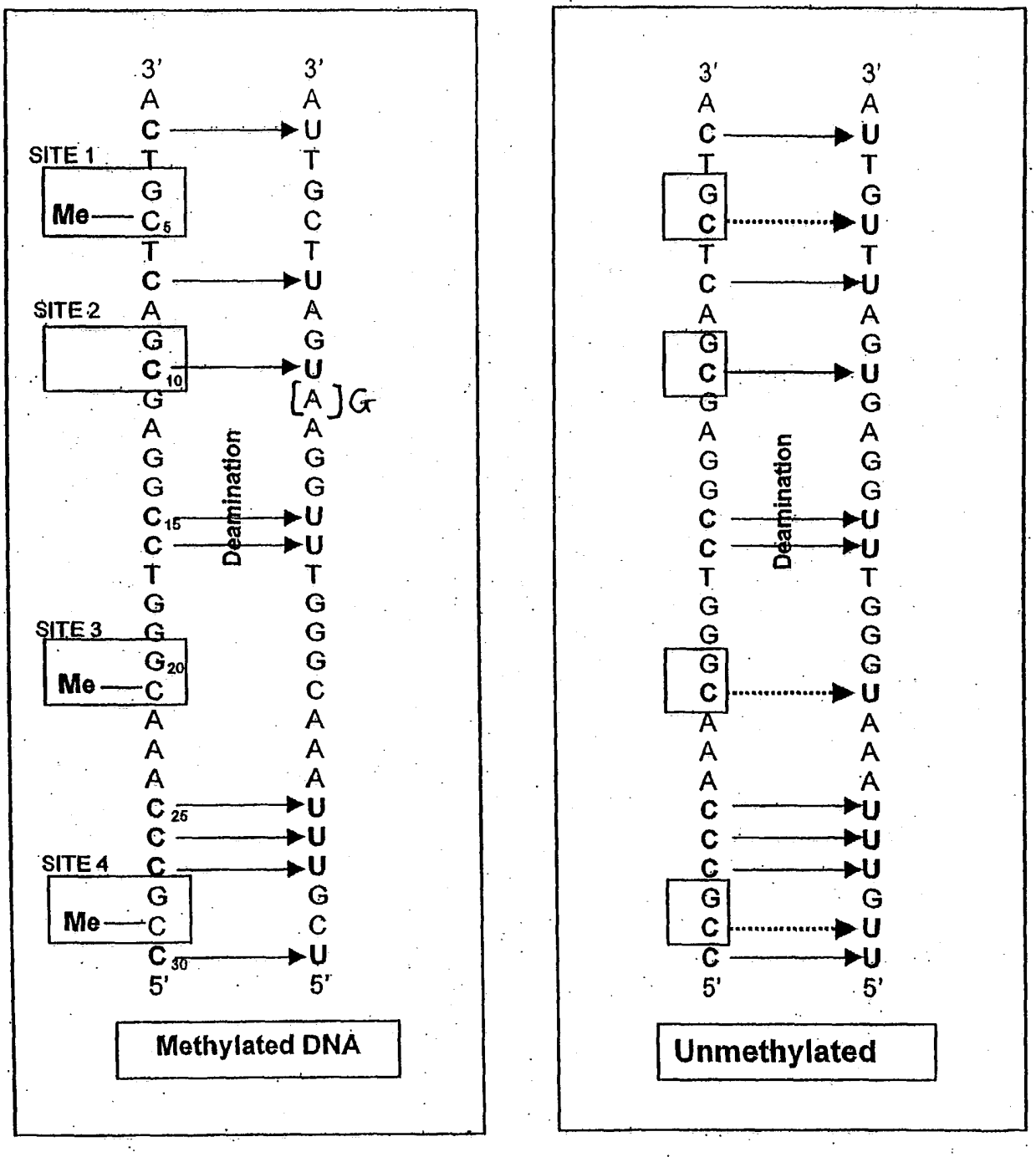

SITE 1
Me
SITE 2
SITE 3
Me
SITE 4
Me
Deamination
Methylated DNA
Deamination
Unmethylated

FIGURE 13

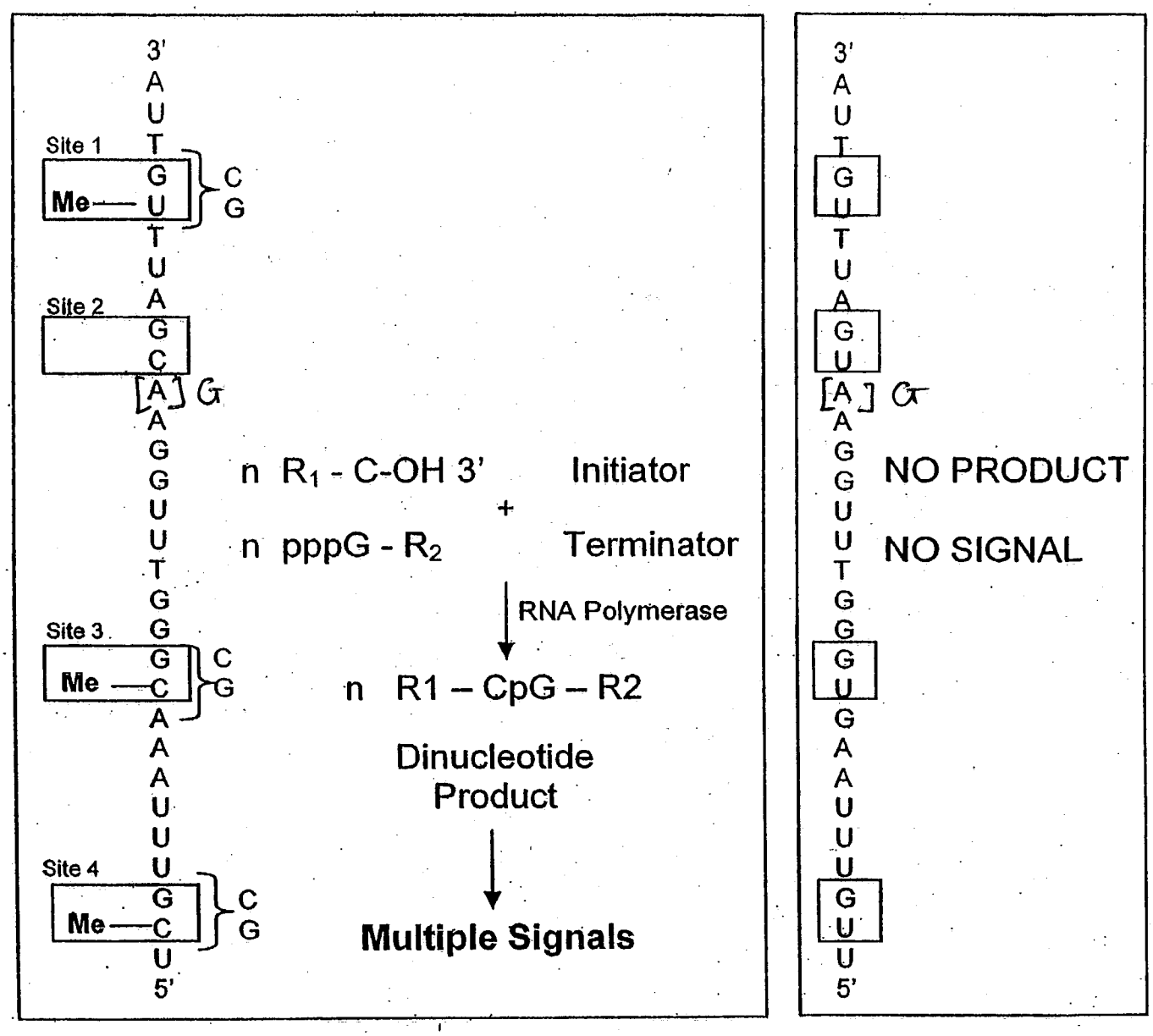

3'
A
U
T
Site 1
G
U
C
G
Me
T
U
A
Site 2
G
C
A
G
n R1 - C-OH 3'
Initiator
+
n pppG - R2
Terminator
RNA Polymerase
G
G
U
U
T
G
G
Site 3
G
C
C
G
Me
n R1 – CpG – R2
A
A
A
U
U
U
Dinucleotide
Product
Site 4
G
C
C
G
Me
Multiple Signals
U
5'
3'
A
U
T
G
U
T
U
A
G
U
A
G
G
U
U
T
G
G
G
U
G
A
A
U
U
U
G
U
U
5'
NO PRODUCT
NO SIGNAL
Deaminated Methylated DNA
Deaminated
Unmethylated
DNA

FIGURE 14

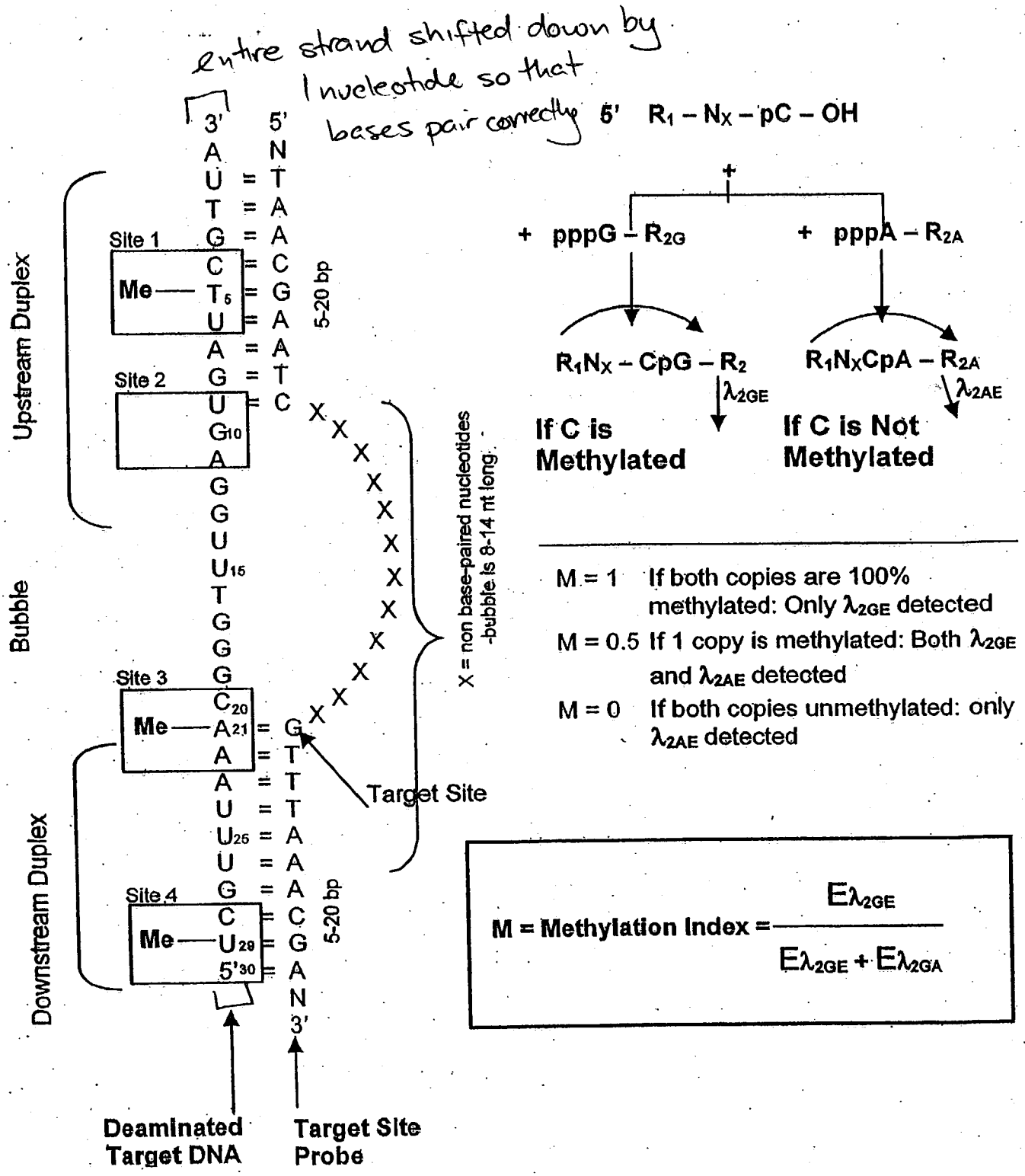

entire strand shifted down by 1 nucleotide so that bases pair correctly
5' R1 – NX – pC – OH
+
+ pppG – R2G
+ pppA – R2A
R1NX – CpG – R2
R1NXCpA – R2A
λ2GE
λ2AE
If C is Methylated
If C is Not Methylated
M = 1 If both copies are 100% methylated: Only λ2GE detected
M = 0.5 If 1 copy is methylated: Both λ2GE and λ2AE detected
M = 0 If both copies unmethylated: only λ2AE detected
M = Methylation Index = Eλ2GE / Eλ2GE + Eλ2GA
Upstream Duplex
Bubble
Downstream Duplex
Site 1
Site 2
Site 3
Site 4
Me
5-20 bp
X = non base-paired nucleotides -bubble is 8-14 nt long.
Target Site
Deaminated Target DNA
Target Site Probe
3'
5'

FIGURE 15

[redacted]ATTATCCAGT
AGGCAGATTAAGCATGTGCTTAAGGCATCAGCAAAGTCTGAGCAATCCATTTTTTAAAACGTAGTACATGTTTT
TGATAAGCTTAAAAAGTAGTAGTCACAGGAAAAATTAGAACTTTTACCTCCTTGCGCTTGTTATACTCTTTAGT
GCTGTTTAACTTTTCTTTGTAAGTGAGGGTGGTGGAGGGTGCCCATAATCTTTTCAGGGAGTAAGTTCTTCTT
GGTCTTTCTTTCTTTCTTTCTTTCTTTTTTTCTTGAGACCAAGTTTCGCTCTTGTCTCCCAGGCTGGAGTGCAA
TGGCGCGATCTCGGCTCACTGCAACCTCCGCCTTCTCCTGGGTTCAAGCGATTCTCCTACATCAGCCTCCGA
GTAGCTGGGATTACAGGCATGCGCCACCAAGCCCCGCTAATTTTGTATTTTTTAGTAGAGACAGGGTTTCGC
CATGTTGGTCAGGCTTGTCTCGAACTCCTGGCCTCAGGTGATCCGCCTGTCTCGGCCTCCCAGAATGCTGG
GATTATAGACGTGAGCCACCGCATCCGGACTTTCCTTTTATGTAATAGTGATAATTCTATCCAAAGCATTTTTT
TTTTTTTTTGAGTCGGAGTCTCATTCTGTCACCCAGGCTGGAGGGTGGTGGCGCGATCTCGGCTTACTGCAA
CCTCTGCCTCCCGGGTTCAAGCGATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGAATTACACACGTGCGCCA
CCATGGCCAGCTAATTTTTGTATTTTTAGTAGAGACGGGGTGTCACCATTTTGGCCAAGCTGGCCTCGAACTC
CTGACCTCAGGTGATCTGCCCGCCTCGGCTTCCCAAAGTGCTGGGATTACAGGTGTGAGCCACCGCGTCCT
GCTCCAAAGCATTTTCTTTCTATGCCTCAAAACAAGATTGCAAGCCAGTCCTCAAAGCGGATAATTCAAGAGC
TAACAGGTATTAGCTTAGGATGTGTGGCACTGTTCTTAAGGCTTATATGTATTAATACATCATTTAAACTCACA
ACAACCCCTATAAAGCAGGGGGCACTCATATTCCCTTCCCCCTTTATAATTACGAAAAATGCAAGGTATTTTC
AGTAGGAAAGAGAAATGTGAGAAGTGTGAAGGAGACAGGACAGTATTTGAAGCTGGTCTTTGGATCACTGTG
CAACTCTGCTTCTAGAACACTGAGCACTTTTTCTGGTCTAGGAATTATGACTTTGAGAATGGAGTCCGTCCTT
CCAATGACTCCCTCCCCATTTTCCTATCTGCCTACAGGCAGAATTCTCCCCCGTCCGTATTAAATAAACCTCA
TCTTTTCAGAGTCTGCTCTTATACCAGGCAATGTACACGTCTGAGAAACCCTTGCCCCAGACAGCCGTTTTAC
ACGCAGGAGGGGAAGGGGAGGGGAAGGAGAGAGCAGTCCGACTCTCCAAAAGGAATCCTTTGAACTAGGG
TTTCTGACTTAGTGAACCCCGCGCTCCTGAAAATCAAGGGTTGAGGGGGTAGGGGGACACTTTCTAGTCGTA
CAGGTGATTTCGATTCTCGGTGGGGCTCTCACAACTAGGAAAGAATAGTTTTGCTTTTTCTTATGATTAAAAGA
AGAAGCCATACTTTCCCTATGACACCAAACACCCCGATTCAATTTGGCAGTTAGGAAGGTTGTATCGCGGAG
GAAGGAAACGGGGCGGGGGCGGATTTCTTTTTAACAGAGTGAACGCACTCAAACACGCCTTTGCTGGCAGG
CGGGGGAGCGCGGCTGGGAGCAGGGAGGCCGGAGGGCGGTGTGGGGGGCAGGTGGGGAGGAGCCCAGT
CCTCCTTCCTTGCCAACGCTGGCTCTGGCGAGGGCTGCTTCCGGCTGGTGCCCCCGGGGGAGACCCAACC
TGGGGCGACTTCAGGGGTGCCACATTCGCTAAGTGCTCGGAGTTAATAGCACCTCCTCCGAGCACTCGCTC
ACGGCGTCCCCTTGCCTGGAAAGATACCGCGGTCCCTCCAGAGGATTTGAGGGACAGGGTCGGAGGGGGC
TCTTCCGCCAGCACCGGAGGAAGAAAGAGGAGGGGCTGGCTGGTCACCAGAGGGTGGGGCGGACCGCGT
GCGCTCGGCGGCTGCGGAGAGGGGGAGAGCAGGCAGCGGGCGGCGGGGAGCAGCATGGAGCCGGCGGC
GGGGAGCAGCATGGAGCCTTCGGCTGACTGGCTGGCCACGGCCGCGGCCCGGGGTCGGGTAGAGGAGGT
GCGGGCGCTGCTGGAGGCGGGGGCGCTGCCCAACGCACCGAATAGTTACGGTCGGAGGCCGATCCAGGT
GGGTAGAGGGTCTGCAGCGGGAGCAGGGGATGGCGGGCGACTCTGGAGGACGAAGTTTGCAGGGGAATT
GGAATCAGGTAGCGCTTCGATTCTCCGGAAAAAGGGGAGGCTTCCTGGGGAGTTTTCAGAAGGGGTTTGTA
ATCACAGACCTCCTCCTGGCGACGCCCTGGGGGCTTGGGAAGCCAAGGAAGAGGAATGAGGAGCCACGCG
CGTACAGATCTCTCGAATGCTGAGAAGATCTGAAGGGGGGAACATATTTGTATTAGATGGAAGTATGCTCTTT
ATCAGATACAAAATTTACGAACGTTTGGGATAAAAAGGGAGTCTTAAAGAAATGTAAGATGTGCTGGGACTAC
TTAGCCTCCAATTCACAGATACCTGGATGGAGCTTATCTTTCTTACTAGGAGGGATTATCAGTGGAAATCTGT
GGTGTATGTTGGAATAAATATCGAATATAAATTTTGATCGAAATTATTCAGAAGCGGCCGGGCGCGGTGCCTC
ACGCCTTGTAATCCCTTCACTTTGGGAGATCAAGGCGGGGGGGAATCACCTGAGGTCGGGAGTTCGAGACCA
GCCTGGCCAACAGGTGAAACCTCGCCTCTACTAAAAATACAAAAAGTAGCCGGGGGGTGGTGGCAGGCGCCT
GTAATCCCAGCTACTCGGGAGGTTGAGGCAGGAGAATCGCTTGAACCCGGGAGGCTGAGGTTGTAGTGAAC
AGCGAGATGGAGCCACTTCACTCCAGCCTGGGTGACAGAGTGAGACTTTGTCGAAAGAAAGAAAGAGAGAA
AGAGAGAGAGAAAAATTATTCAGAAGCAACTACATATTGTGTTTATTTTTAACTGAGTAGGGCAAATAAATATA
TGTTTGCTGTAGGAACTTAGGAAATAATGAGCCACATTCATGTGATCATTCCAGAGGTAATATGTAGTTACCAT
TTTGGGAATATCTGCTAACATTTTTGCTCTTTTACTATCTTTAGCTTACTTGATATAGTTTATTTGTGATAAGAG
TTTTCAATTCCTCATTTTTGAACAGAGGTGTTTCTCCTCTCCCTACTCCTGTTTTGTGAGGGAGTTAGGGGAG
GATTTAAAAGTAATTAATACATGGGTAACTTAGCATCTCTAAAATTTTGCCAACAGCTTGAACCCGGGAGTTTG
GCTTTGTAGTCCTACAATATCTTAGAAGAGACCTTATTTGTTTAAAAACAAAAAGGAAAAAGAAAAGTGGATAG
TTTTGACAATTTTTAATGGAG

Figure 30

29

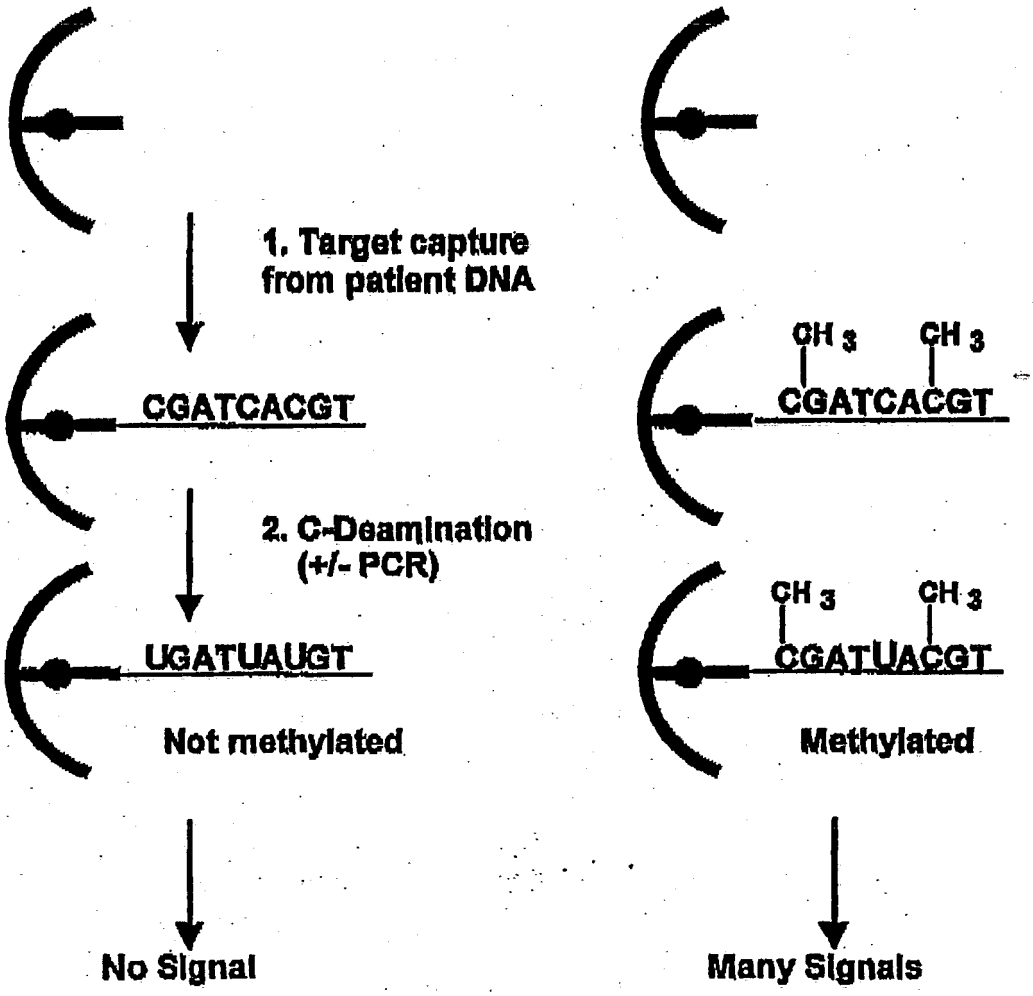

1. Target capture
from patient DNA
CGATCACGT
2. C-Deamination
(+/- PCR)
UGATUAUGT
Not methylated
No Signal
CH 3
CH 3
CGATCACGT
CH 3
CH 3
CGATUACGT
Methylated
Many Signals

FIGURE [31]